

[B] Normal forms in acid media	[II] No acid from starch
(1) Surface growth in stab somewhat expanded and flat	[B] Acid from sucrose but not from maltose
(a) Colonies brownish red	— No. 13 — (2) No acid from arabinose (acid from glycerol and erythritol)
(2) Surface growth in stab small and dome shaped	[C] No acid from sucrose or maltose
(a) Colonies cream colored	— No. 6 — (1) Acid from arabinose
[II] Small streptococci	
[A] Normal forms in acid media	
(1) Surface growth in stab somewhat expanded and flat	
(a) Colonies cream colored	— No. 1 — (2) No acid from arabinose (No acid from glycerol or erythritol)
[B] Irregular forms in acid media	
(1) Surface growth in stab scanty	[A] Acid from sucrose and maltose
(a) Colonies cream colored	— No. 14 — (1) Acid from arabinose

The distinct separation of these strains from the previously described species may be seen from the fact that the following supplementary modifications proved to be necessary to fit these new strains into the keys for the identification of the species of *Propionibacterium* presented by Werkmann⁽²⁾ and Van Niel⁽¹⁾. The supplemented parts are italicised in the following keys:

The key by Werkmann and Brown⁽²⁾ (modified by the authors to include the new strains).

A. Attacking sucrose and maltose	
B. Attacking polysaccharides	
C. Attacking lactose and arabinose	
D. Attacking rhamnose and trehalose, not attacking raffinose and xylose, catalase negative	—Propionibac. arabinosum
DD. Not attacking rhamnose, trehalose and xylose, attacking raffinose, catalase positive	—Propionibac. technicum
DDD. Not attacking raffinose, attacking xylose, catalase positive	
E. Colonies on lactate media orange coloured	—No. 11
EE. Colonies on lactate media not orange coloured	—No. 3
CC. Not attacking lactose and arabinose	—Propionibac. zae
BB. Not attacking polysaccharides	
C. Attacking xylose and arabinose, nitrates reduced	—Propionibac. pentosaceum
CC. Not attacking xylose and arabinose, nitrates not reduced	
D. Attacking raffinose	
E. Pigment yellow	—Propionibac. raffinosaceum
EE. Pigment red brown	—Propionibac. rubrum
DD. Not attacking raffinose	
E. Attacking mannitol, not attacking sorbitol	
F. Attacking amygdalin and salicin	—Propionibac. Peterssonii
FF. Not attacking amygdalin and salicin	—Propionibac. Jensenii
EE. Not attacking mannitol, attacking sorbitol	—Propionibac. Thoenii
CCC. Not attacking xylose, attacking arabinose, nitrates not reduced	—No. 4 (streptococci)
AA. Not attacking sucrose and maltose	
B. Attacking lactose, nitrates not reduced	—Propionibac. Shermanii (streptococci)

BB. Not attacking lactose, nitrates reduced	— Propionibac. Freudenreichii (<i>streptococci</i>)
BBB. Attacking lactose, nitrates reduced	— No. 6 (short rods)
AAA. Attacking sucrose, not attacking maltose, nitrates reduced	
B. Attacking glycerol and erythritol	— No. 13 (short rods)
BB. Not attacking glycerol and erythritol	— No. 1 (<i>streptococci</i>)

The key by Van Niel⁽¹⁾ (modified by the authors to include the new strains).

- I. In yeast extract dextrose media growth occurs in the form of small streptococci. Dirty cream-colored growth in stabs, with slight surface growth of same color. Sucrose and maltose not fermented.
 - A. Not fermenting lactose — Propionibact. Freudenreichii
 - AA. Fermenting lactose — Propionibact. Shermanii
- I I. In yeast extract dextrose media growth occurs in the form of small streptococci. Irregular elongated cells in acid media. Dirty cream-colored growth in stabs, with slight surface growth. Sucrose and maltose are fermented.
 - No. 4
- I I I. In yeast extract dextrose media growth occurs in the form of small streptococci. No variation of cell form in acid media. Dirty cream colored growth in stabs, with expanded and flat surface growth. Sucrose is fermented but not maltose.
 - No. 1
- II. In yeast extract dextrose media growth occurs in the form of typical short rods of diphtheroid appearance. Distinct surface growth in stabs. Sucrose and maltose are fermented.
 - A. Growth brownish red
 1. Ferments raffinose and mannitol, but not sorbitol — Propionibac. rubrum
 2. Ferments sorbitol, but not raffinose and mannitol — Propionibac. Thoenii
 - B. Growth in stab cream colored
 1. Surface growth cream colored
 - a. Ferments *l*-arabinose and rhamnose — Propionibac. zaeae
 2. Surface growth yellow to orange
 - a. Growth in liquid media flocculent, as if agglutinated — Propionibac. Peterssonii
 - aa. Growth in liquid media dispersed, smooth
 - b. Do not ferment dextrin, glycogen and starch
 - bb. Ferments dextrin, glycogen and starch
 - Propionibac. technicum
 - No. 11 (?) and No. 3 (?)
 - II II. In yeast extract dextrose media growth occurs in the form of typical short rods of diphtheroid appearance. Distinct surface growth in stab. Sucrose and maltose are not fermented.
 - No. 6
 - II II II. In yeast extract dextrose media growth occurs in the form of typical short rods of diphtheroid appearance. Surface growth somewhat expanded and flat. Ferments sucrose but not maltose. Growth brownish red.
 - No. 13
 - III. In yeast extract dextrose media growth occurs in the form of highly irregular cells, giving the appearance of involution forms. Distinct surface growth in stabs. Both *l*-and *d*-arabinose are fermented.
 - A. Involution forms large, swollen spheres, surface growth orange yellow,

does not ferment xylose and rhamnose — Propionibac. arabinosum
 B. Involution forms long, irregular rods. Surface growth cream colored.
 Ferments xylose and rhamnose — Propionibac. pentosaceum
 — No. 3 (?)
 BB. Involution forms long, irregular rods. Surface growth orange yellow.
 Ferments xylose. — No. 11 (?)

The properties of the isolated bacteria.

(1) *Propionibacterium globosum* nov. sp.

Culture. No. 1.

Morphology. In sodium lactate broth at 30°C, $0.5\ \mu \times 0.6\ \mu$, single coccus, occasionally in chains, no metamorphosis in acid media, assuming long forms, $0.6\ \mu \times 3\sim 5\ \mu$, under aerobic conditions. Non-motile. Spore not formed. Gram-positive.

Cultural characteristics. Liquid media: No or little turbidity; cream-colored, somewhat flocculent sediment.

Agar stab: Both in sodium lactate and glucose bouillon agar, growths are abundant with stretched surface growth. No pigment, creamy.

Litmus milk: completely decolorized, acid, coagulated.

Physiology. Catalase positive, nitrates reduced to nitrites, indol negative, H_2S not produced and gelatin not liquefied. Optimum temperature for acid production $33^\circ \sim 34^\circ C$. Optimum pH 7.0. Killed at 75°C, in 10 minutes. Acid from fructose, glucose, galactose, mannose, lactose and saccharose. No acid from xylose, arabinose, maltose, raffinose, dextrin, starch, inulin, dulcitol, mannitol, glycerol, erythritol, salicin and amygdalin.

Propionic and acetic acids are produced from glucose in the ratio of about 2 : 1.

(2) *Propionibacterium amylaceum* nov. sp.

Cultures. No. 3 (9 and 10).

Morphology. In sodium lactate broth at 30°C, the cells appear as short rods, $0.6\ \mu \times 1.2\sim 1.5\ \mu$, in acid media long irregular cells, $0.6\ \mu \times 7\sim 8\ \mu$. Under aerobic conditions, straight shorter rods, about $0.6\ \mu \times 4\sim 5\ \mu$. Non-motile. Spore not formed. Gram positive.

Liquid media: Distinctly turbid with grayish-creamy,ropy sediment.

Agar stab: both in sodium lactate and glucose bouillon agar pin head shaped small surface growth, abundant ropy stab growth. Gas production. No pigment.

Litmus milk completely decolorized, acid and coagulated.

Physiology. Catalase positive, nitrates not reduced, indol negative, H_2S not liquefied.

Optimum temperature for acid production $30^\circ \sim 34^\circ C$. Optimum pH 6.8. Killed at 70°C in 10 minutes. Acid from xylose, arabinose, fructose, glucose, galactose, maltose, mannose, lactose, saccharose, dextrin, starch, mannitol, glycerol, erythritol, salicin and amygdalin.

No acid from raffinose, dulcitol and inulin.

Propionic and acetic acids are produced from glucose in the ratio of about 4.7 : 1.

(3) *Propionibacterium japonicum* nov. sp.

Cultures. No. 4 (and 5).

Morphology. In sodium lactate broth at 30°C, spherical, $0.6 \mu \times 0.6 \mu$; in acid media cells appear as long rods, $0.6 \mu \times 8 \mu$. Non-motile. Spore not formed. Gram positive.

Liquid media: No or little turbidity; cream colored, flocculent sediment.

Agar stab: both in sodium lactate and glucose bouillon agar, growths slight, with little or no surface growth. No pigment.

Litmus milk: decolorized but not coagulated.

Physiology. Nitrates not reduced to nitrites, indol negative, H_2S not produced, gelatin not liquefied.

Produce little or no catalase power.

Optimum temperature for acid production 30°C, optimum pH 7.0~7.2.

Killed at 70°C in 10 minutes. Acid from arabinose, fructose, glucose, galactose, maltose, mannose, lactose, saccharose, dextrin, mannitol, salicin and amygdalin.

No acid from xylose, raffinose, starch, dulcitol, inulin, glycerol and erythritol.

Propionic and acetic acids are produced from glucose in the ratio of about 2.2 : 1.

(4) *Propionibacterium orientum* nov. sp.

Cultures. No. 6 (8₁ and 8₂).

Morphology. In sodium lactate broth at 30°C, the cells appear as short rods, $0.5 \sim 0.6 \mu \times 1.0 \sim 1.2 \mu$, no metamorphosis in acid media, assuming long forms, $0.6 \times 4 \sim 5 \mu$, under aerobic conditions. Non-motile. Spore not formed. Gram positive. Liquid media: Distinctly turbid; smooth creamy sediment.

In agar cultures, both in sodium lactate and glucose bouillon agar, growths abundant, "beads-connected," with moderate surface growth.

Gas produced. Pigment: slight yellowish, if present.

Litmus milk: decolorized, acid, coagulated.

Physiology. Catalase positive; nitrates reduced to nitrites; indol negative; H_2S not produced; gelatin not liquefied.

Optimum temperature for acid production, 34°C. Optimum pH 7.0~7.2.

Killed at 75°C in 10 minutes. Acid from arabinose, fructose, glucose, galactose, mannose, lactose, glycerol and erythritol.

No acid from xylose, maltose, saccharose, raffinose, dextrin, starch, dulcitol, inulin, mannitol, salicin and amygdalin.

Propionic and acetic acids are produced from glucose in the ratio of about 1.8 : 1.

(5) *Propionibacterium amylaceum* nov. sp. var. *aurantium* nov. var.

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Culture. No. 11. *second report* *to the editor* *for this article* *has been accepted*

Morphology. In sodium lactate broth at 30°C, short rods, $0.6 \mu \times 1.2 \sim 1.5 \mu$;

in acid media, irregular long form $0.6 \times 7 \sim 8 \mu$; assuming straight rods, $0.6 \mu \times 4 \sim 5 \mu$, under aerobic conditions. Non-motile. Spore not formed.

Gram positive.

Liquid media: Distinctly turbid with grayish-creamy, ropy sediment.

Agar stab: both in sodium lactate and glucose bouillon agar, growths are abundant and ropy, with pin-head sized moderate surface growth.

Gas produced. Orange yellow pigment in sodium lactate medium.

Litmus milk: Completely decolorized, acid, coagulated.

Physiology. Catalase positive, nitrates not reduced to nitrites. Indol negative, H_2S not produced. Gelatin not liquefied.

Optimum temperature for acid production, 30°C. Optimum pH 5.6~6.8.

Killed at 70°C in 10 minutes. Acid from xylose, arabinose, fructose, glucose, galactose, maltose, mannose, lactose, saccharose, dextrin, starch, mannitol, glycerol, erythritol, salicin and amygdalin.

No acid from inulin, dulcitol and raffinose.

Propionic and acetic acids are produced from glucose in the ratio of about 3.8 : 1.

(6) *Propionibacterium coloratum* nov. sp.

Cultures. No. 13. (and 1₂)

Morphology. In sodium lactate broth at 30°C, short rods, $0.6 \mu \times 1.0 \sim 1.2 \mu$, no metamorphosis in acid media, assuming long forms, $0.6 \mu \times 3 \sim 5 \mu$, under aerobic conditions. Non-motile. Spore not formed. Gram positive.

Liquid media: Distinctly turbid in early stages, ropy sediment.

Stab culture: Both in glucose and sodium lactate bouillon agar, growths are moderate and ropy with somewhat stretched surface growth.

Gas produced.

Litmus milk: complete decolorization, acid, coagulation.

Physiology. Catalase positive, nitrates reduced to nitrites, indol negative, H_2S not produced, gelatin not liquefied.

Optimum temperature for acid production 34°C, optimum pH 6.5~6.8.

Killed at 75°C in 10 minutes.

Acid from fructose, glucose, galactose, mannose, lactose, saccharose, glycerol and erythritol. No acid from xylose, arabinose, maltose, raffinose, dextrin, starch, inulin, dulcitol, mannitol salicin and amygdalin.

Propionic and acetic acids are produced from glucose in the ratio of about 2.5 : 1.

(1) Bergey's Manual of Determinative Bacteriology, 5th Ed.

(2) Werkmann and Brown: Journ. Bact. 26, 400, 1933.

Über eine neue Quantitative Analyse des Eisens.

(SS. 139~143)

Von Shinichiro BABA.

(Aus dem Agrikulturchem. Laboratorium der Kaiserl. Universität Tokio;

Eingegangen am 27. 1. 1941.)

Dieses Verfahren bezweckt, eine einfache Massanalyse zu finden, in der die Farbenreaktion des Ferrisalzions durch gelbes Blutlaugensalz verwendet wird.

Die Farbenreaktion durch das gelbe Blutlaugensalz ist sehr scharf, und in Ansäuerung der Schwefelsäure wird es bis zu etwa 1/10000 gefärbt, weshalb der Anwendungsumfang dieses Verfahrens auf dieses Mass beschränkt ist.

Der Gehalt des Eisens in der Laugeprobe soll nicht mehr als 1 mgr. sein.

Durch dieses Verfahren gewinnt man eine kleinere Menge als durch die Gewichtsanalyse. Die Differenz beträgt -8.4 %.

Ammoniumsulfat und Ferrosalz stören die Ausführung dieses Verfahrens. Deshalb muss das Ferrosalz durch Salpetersäure zum Ferrisalz umgewandelt werden.

Bei den Substanzen, welche die Azidität durch die Oxydation anzeigen, kann die Differenz der Titrierung durch das Neutralrot beseitigt werden.

Für die freundliche Hilfe bei dieser Untersuchung sage ich an dieser Stelle meinen verbindlichsten Dank den Herren Prof. Dr. K. Sakaguchi, a. o. Professor Dr. T. Asai, a. o. Professor Fujihara, den Herren des Seminars und Herrn Ozaki.

On the Brown Forest Soil in the Upper Region of the Non River, North Manchuria.

(pp. 144~148)

By R. KAWASHIMA and M. NAGATA.

(Agricultural Chemical Laboratory, Kyushu Imperial University;

Received February 1, 1941.)

A brown forest soil of good quality is widely distributed along the upper course of the Non River of North Manchuria. The parent material of the soil is diluvial deposit. The soil texture is composed of fine clay and the A₁ layer contains a good quantity of humus.

For the region now concerned is exhibited the functional relationship between humidity and soil properties. The mean annual temperature in this region does not differ much and the annual precipitation increases from south to north, and so the humidity is increasing in that direction.

In company with the increasing humidity, the following correlations are observed :

- i Both the clay and nitrogen contents increase.
- ii Both the pH-values and degrees of base saturation decrease.
- iii The total exchange capacities increase.

In an appended map in Thorp's book⁽¹⁾, the soil type of this region is recorded as a chernozem, but this is a mistake.

(1) J. Thorp: *Geography of the Soil of China*, 1936. (Nanking)

On the Preparation and Some Properties of Yeast Amylase.

(pp. 149~152)

By Reitaro MURAKAMI.

(Utunomiya Agricultural College; Received February 7, 1941.)

Functional Studies on Soils. (XI~XII).

(pp. 153~160)

By MISU-Hideo.

(Agricultural Experiment Station, Government General of Tyōsen;
Received January 11, 1941.)

Ein synthetischer Versuch von β -Indol-essigsäure (Indol-3-essigsäure).

(SS. 161~164)

Von Sankiti TAKEI u. Takenosuke TAKANO.

(Aus d. Institut f. Chem. Forschung, Kaiserl. Universität Kyoto;
Eingegangen am 11. 2. 1941.)

On the Fermentation Degumming of Waste Silk. Part VI.

The Action of Degummase and Thermodegummase upon Protein Substances.

(pp. 165~170)

By Hideo KATAGIRI and Toshio NAKAHAMA.

(Agricultural Chemical Laboratory, Kyoto Imperial University)

Received February 26, 1941.)

It was ascertained in the previous paper that the active centre of the useful bacteria *BACILLUS CEREUS* and *BACILLUS ROBUSTUS* for fermentation degumming was due to soluble enzymes named degummase and thermodegummase respectively.

The preparations of these enzymes were obtained in the following manner: the cultural solution was evaporated under reduced pressure after being dialysed with collodium membrane, and precipitated by acetone.

With these preparations, all the protein substances: glycyl glycine, chloracetyl *l* tyrosin, leucyl diglycine, peptone, gelatin, egg albumin, sericin, edestin and casein, were attacked. With degummase (op. pH=6.0, op. temp.=40°) glycyl glycine and casein were readily decomposed and edestin was slightly attacked, while very remarkable decomposition of gelatin and edestin was observed with thermodegummase (op. pH=7.5, op. temp.=55°).

It was therefore reasonable that these bacteria were useful for the degumming of waste silk, since degummase and thermodegummase were composed of protease system including proteinase, aminopolypeptidase, dipeptidase and carbonylpeptidase.

Researches on "Maoran" as a Raw Material for Paper Pulp and Rayon Pulp.

(pp. 171~191)

By Motô YAMANE and Tomizô MATUI.

(Chemical Laboratory, Hukukuseni Kogyo; Received January 28, 1941.)

In this paper, the researches on chemical components and cooking experiments of "Maoran" (New Zealand flax, *Phormium tenax*) are described.

On the Fixation of Silk-Sericin with Formaldehyde.

(pp. 192~196)

By Tosio NAKAHAMA and Ikuzo SAKAGUCHI.

(Kanebo Yamashina Institute; Received February 3, 1941.)

On the fixation of silk-sericin with formaldehyde solution, we have first studied the optimum concentration, temperature and pH of the aldehyde solution, and then the optimum period of treatment and the influence of sodium chloride.

The experimental results are summarized as follows:

(1) The optimum conditions of aldehyde solution for the fixation of sericin were found to be

Concentration.....	4 %	Period of treatment.....	3 hour
Temperature	50°C	pH	about 7

(2) The adsorption phenomenon of formaldehyde on natural silk was observed according to the formula of Freundlich's adsorption isotherm using a dilute solution of less than 4 % form aldehyde.

(3) Sodium chloride did not reveal any remarkable influence on the fixation of sericin with aldehyde.

Biochemical Studies on Diphtheria Toxin.

(2nd report)

(pp. 197~198)

By Tetutaro TADOKORO, Tuneyuki SAITO

and Naomoto TAKASUGI.

(Hokkaido Imperial University; Received February 12, 1941.)

On the Hydrolysis of Fats and Fatty Acid Esters. (9).

(pp. 199~209)

By Toyoki ONO.

(Chemical Laboratory of the Fish Meal Association of Japan;

Received February 14, 1941)

Relation between the Unsaturation of Oils and their Hydrolyses.

Some authors have reported that the velocity of hydrolysis of fats and oils is influenced by their unsaturation. In previous paper, however, I have described that these facts are not observed at ordinary temperature, but the higher unsaturated oils have greater velocity of hydrolysis than the lower unsaturated or saturated ones at such a low temperature as -4°C.

To solve those problems the following experiments were carried out, and my previous works were perfectly proved by these results.

EXPERIMENTS and RESULTS.

Eight samples were taken as the higher unsaturation oils (perilla, linseed and sardine oils), the lower unsaturated oils (olive and soya bean oils) and saturated oils (cacao butter, cocoa nut oil and lard).

The velocity of hydrolysis was determined at 30°, 0° and -4°C with pancreas and ricinus lipase. The results are summarised in Table 8.

(1). As in my previous works, it was distinctly observed that there is no relation between the hydrolysis and the unsaturation at 30°C.

(2). At 0° and -4°C, however, the velocity of hydrolysis diminishes in order of the higher unsaturated, the lower unsaturated and saturated oils.

(3). Such a fact on hydrolysis at lower temperature is due to the physico-chemical properties, mainly the surface tension, of emulsions. Table 9 shows this explanation.

Table 8. Influence of the Unsaturation of Fats and Oils on their Hydrolysis.

Fat and oils	Iodine number	Pancreas lipase		Ricinus lipase					
		30°C		30°C		0°C		-4°C	
		After 1 hr.	After 3 hrs.	After 1 hr.	After 3 hrs.	After 20 hrs.	After 40 hrs.	After 20 hrs.	After 65 hrs.
Cocoa nut oil	8.43	28.83	42.66	18.55	32.05	4.07	5.40	—	—
Cacao butter	37.40	26.72	35.52	10.00	17.76	—	—	—	—
Lard	56.12	18.07	23.74	6.41	11.13	2.29	6.21	—	—
Olive oil	88.47	21.33	32.64	23.28	39.84	12.84	21.64	33.77	48.70
Soya bean oil	137.00	20.83	31.31	19.42	32.12	—	—	—	—
Sardine oil	175.94	22.02	30.13	13.85	19.83	11.93	27.80	—	—
Linseed oil	183.32	24.71	37.94	23.83	36.03	19.40	53.40	—	—
Perilla oil	187.03	26.66	37.61	22.74	35.42	26.73	51.30	49.53	66.13

Table 9. Relation between the Surface Tension of Emulsions and Temperature.

Temp.	Water	Emulsion I				Emulsion II			
		Olive oil		Perilla oil		Olive oil		Perilla oil	
		h	h	σ	h	σ	h	σ	h
30°C	30.5	10.5	21.7	9.0	18.9	12.8	26.5	12.0	25.3
18	30.5	10.7	22.3	9.0	19.1	12.5	26.0	11.7	24.8
0	30.0	8.0	16.6	8.9	19.0	11.0	23.2	11.6	24.8
-4	—	0°	—	8.8	19.0	—	—	—	—

h represent the hight in mm. of emulsion rising capillary of 0.5 mm diameter, and σ the surface tension.

Some Experiments on Fresh Tobacco Leaves.

(pp. 210~218)

By K. Ōike.

(Central Research Institute, Japanese Government Monopoly Bureau;

Received February 26, 1941.)

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